A Genome-Wide Association Study of Depressive Symptoms

Karin Hek\textsuperscript{a}, Ayse Demirkan\textsuperscript{a}, Jari Lahti\textsuperscript{b}, Antonio Terracciano\textsuperscript{c}, Alexander Teumer\textsuperscript{d}, Marilyn C. Cornelis\textsuperscript{e}, Najaf Amin\textsuperscript{e}, Erin Bakshis\textsuperscript{f}, Jens Baumert\textsuperscript{g}, Jingzhong Ding\textsuperscript{h}, Yongmei Liu\textsuperscript{i}, Kristin Marcianti\textsuperscript{j}, Osorio Meirelles\textsuperscript{k}, Michael A. Nalls\textsuperscript{l}, Yan V. Sun\textsuperscript{m}, Nicole Vogelzangs\textsuperscript{n}, Lei Yu\textsuperscript{o}, Stefania Bandinelli\textsuperscript{p}, Emelia J. Benjamin\textsuperscript{q}, David A. Bennett\textsuperscript{r}, Dorret Boomsma\textsuperscript{s}, Alessandra Cannas\textsuperscript{t}, Laura H. Coker\textsuperscript{u}, Eco de Geus\textsuperscript{v}, Philip L. De Jager\textsuperscript{w}, Ana V. Diez-Roux\textsuperscript{x}, Shaun Purcell\textsuperscript{y}, Frank B. Hu\textsuperscript{z}, Eric B. Rimma\textsuperscript{aa}, David J. Hunter\textsuperscript{ab}, Majken K. Jensen\textsuperscript{ac}, Gary Curhan\textsuperscript{ad}, Kenneth Rice\textsuperscript{ae}, Alan D. Penman\textsuperscript{af}, Jerome I. Rotter\textsuperscript{ag}, Nona Sotoodehnia\textsuperscript{ah}, Rebecca Emeny\textsuperscript{ai}, Johan G. Eriksson\textsuperscript{aj}, Denis A. Evans\textsuperscript{ak}, Luigi Ferrucci\textsuperscript{al}, Myriam Fornage\textsuperscript{am}, Vilmundur Gudnason\textsuperscript{an}, Albert Hofman\textsuperscript{ao}, Thomas Illig\textsuperscript{ap}, Sharon Kardia\textsuperscript{aq}, Margaret Kelly-Hayes\textsuperscript{ar}, Karestan Koenen\textsuperscript{as}, Peter Kraft\textsuperscript{at}, Maris Kuningas\textsuperscript{au}, Joseph M. Massaro\textsuperscript{av}, David Melzer\textsuperscript{aw}, Antonella Mulas\textsuperscript{ax}, Cornelis L. Mulder\textsuperscript{ay}, Anna Murray\textsuperscript{az}, Ben A. Oostra\textsuperscript{ba}, Aarno Palotie\textsuperscript{bb}, Brenda Penninx\textsuperscript{bc}, Astrid Petersmann\textsuperscript{bd}, Luke C. Pilling\textsuperscript{be}, Bruce Psaty\textsuperscript{bf}, Rajesh Rawal\textsuperscript{bg}, Eric M. Reiman\textsuperscript{bh}, Andrea Schulz\textsuperscript{bi}, Joshua M. Shulman\textsuperscript{bj}, Andrew B. Singleton\textsuperscript{bk}, Albert V. Smith\textsuperscript{bl}, Angelina R. Sutin\textsuperscript{bm}, André G. Uitterlinden\textsuperscript{bn}, Henry Völzke\textsuperscript{bo}, Elisabeth Widen\textsuperscript{bp}, Kristine Yaffe\textsuperscript{bq}, Alan B. Zonderman\textsuperscript{br}, Francesco Cucca\textsuperscript{bs}, Tamara Harris\textsuperscript{bt}, Karl-Heinz Ladwig\textsuperscript{bu}, David J. Llewellyn\textsuperscript{bv}, Katri Räikkönen\textsuperscript{bw}, Toshiko Tanaka\textsuperscript{bx}, Cornelia M. van Duijn\textsuperscript{by}, Hans J. Grabe\textsuperscript{bz}, Lenore J. Launer\textsuperscript{ca}, Kathryn L. Lunetta\textsuperscript{cb}, Thomas H. Mosley Jr.\textsuperscript{cc}, Anne B. Newman\textsuperscript{cd}, Henning Tiemeier\textsuperscript{ce}, and Joanne Murabito\textsuperscript{cf}

\textsuperscript{a}Research Centre O3 (KH, CLM, HT), Department of Psychiatry, and Department of Epidemiology (KH, AH, MK, AGU, HT), Erasmus MC, Rotterdam, The Netherlands; Institute of Behavioural Sciences (JL, KRa), University of Helsinki, Helsinki, Finland; Clinical Research Branch (TT), National Institute on Aging, Baltimore, Maryland; Interfaculty Institute for Genetics and Functional Genomics (ATEu), University Medicine Greifswald, Greifswald, Germany; Department of Nutrition (MCC, FBH, EBR, MKJ), Harvard School of Public Health, Boston, Massachusetts; Department of Epidemiology (EB, AVD-R, SK), University of Michigan School of Public Health, Ann Arbor, Michigan; Institute of Epidemiology II (JB, RE, K-HL), Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany; Department of Internal Medicine (JD), Division of Geriatrics, and Department of Epidemiology and Prevention (YL), Division of Public Health Sciences, Wake Forest University, Winston-Salem, North Carolina; Department of Medicine (KM), Cardiovascular Health Research Unit, University of Washington, Seattle, Washington; National Institute on Aging (ATer, OM, ARS), National Institutes of Health, Department of Health and Human Services, Baltimore; and Laboratory of Neurogenetics (MAN, ABS), Intramural Research Program, National Institute on Aging, National Institutes of Health, Bethesda, Maryland; Department of Epidemiology (YVS), Emory University, Atlanta, Georgia; EMGO Institute for Health and Care Research (NV, BPe), and Department of Psychiatry (NV, BPe), VU University Medical Center, Amsterdam, The Netherlands; Rush Alzheimer’s Disease Center (LY, DAB), and Department of Neurological Sciences (LY, DAB), Rush University Medical Center, Chicago, Illinois; Geriatric Unit Azienda Sanitaria di Firenze (SB), Firenze, Italy; National Heart, Lung, and Blood Institute’s Framingham Heart Study (EJB, MK-H, JMM, KLL, JM), Framingham; and Department of Medicine (EJB), Section of Cardiology and Preventive Medicine, Boston University School of Medicine and Public Health, Boston, Massachusetts; Department of
Institute on Aging, Bethesda, Maryland; Department of Biostatistics (KLL), Boston University School of Public Health, Boston, Massachusetts; Department of Medicine (THM), University of Mississippi Medical Center, Jackson, Mississippi; Department of Medicine (JM), Section of General Internal Medicine, Boston University School of Medicine; Program in Molecular and Genetic Epidemiology (DJH, PK), Harvard School of Public Health; and Brigham and Women’s Hospital (GC), Harvard Medical School, Boston, Massachusetts; Department of Epidemiology (ABN), Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania; Department of Medicine (VG, AVS), University of Iceland, Reykjavik, Iceland; Hannover Unified Biobank (TI), Hannover Medical School, Hannover, Germany; College of Medicine (ATer, ARS), Florida State University, Tallahassee, Florida; Genetic Epidemiology Unit (AD, NA, BAO, CMvD), Departments of Epidemiology and Clinical Genetics, Erasmus MC, Rotterdam, The Netherlands; and Departments of Neurology and Molecular Human Genetics (JMS), Baylor College of Medicine, Houston, Texas.

These authors contributed equally to this work.

Abstract

Background—Depression is a heritable trait that exists on a continuum of varying severity and duration. Yet, the search for genetic variants associated with depression has had few successes. We exploit the entire continuum of depression to find common variants for depressive symptoms.

Methods—In this genome-wide association study, we combined the results of 17 population-based studies assessing depressive symptoms with the Center for Epidemiological Studies Depression Scale. Replication of the independent top hits (p < 1 × 10^{-5}) was performed in five studies assessing depressive symptoms with other instruments. In addition, we performed a combined meta-analysis of all 22 discovery and replication studies.

Results—The discovery sample comprised 34,549 individuals (mean age of 66.5) and no loci reached genome-wide significance (lowest p = 1.05 × 10^{-7}). Seven independent single nucleotide polymorphisms were considered for replication. In the replication set (n = 16,709), we found suggestive association of one single nucleotide polymorphism with depressive symptoms (rs161645, 5q21, p = 9.19 × 10^{-3}). This 5q21 region reached genome-wide significance (p = 4.78 × 10^{-8}) in the overall meta-analysis combining discovery and replication studies (n = 51,258).

Conclusions—The results suggest that only a large sample comprising more than 50,000 subjects may be sufficiently powered to detect genes for depressive symptoms.

Keywords

Center for Epidemiologic Studies Depression Scale; CHARGE consortium; depression; depressive symptoms; genetics; genome-wide association study; meta-analysis

Major depressive disorder (MDD) is a complex disease with an underlying heritable component. Family and twin studies report a high familial tendency of the disorder and heritability estimates of 31% to 42% (1,2). However, the long search for genetic variants associated with depression has had few successes. Several linkage studies for major depressive disorder have been performed and these identified only one relevant locus (3,4). In addition, hundreds of candidate genes have been investigated in association studies, but only six variants have been confirmed in meta-analyses (5,6). Recent efforts to find new candidate genes via genome-wide association studies (GWAS) have also been largely unsuccessful (7–15). Genome-wide association studies identified interesting regions, but associations with MDD reached standard levels of genome-wide significance at only one locus (15). Furthermore, only few previously reported candidate genes were replicated in genome-wide association studies (7,13,16).
Depression exists on a continuum of varying severity and duration. Depressive symptoms (measured on a continuous scale) and MDD (measured on a dichotomous scale) are associated with similar patterns of risk factors suggesting shared etiology with varying severity (17). The ability to detect genetic predictors might, therefore, be improved by analyzing depression quantitatively (18), defining MDD as a diagnostic entity applied to the extreme of the depression continuum (19). Using the phenotypic variation within cases and control subjects by analyzing depression quantitatively has been shown to greatly increase the power to detect genetic variants (20). In fact, a GWAS of the depression facet of personality (a continuous trait) identified several candidate genes. However, the sample size was small and findings remain to be confirmed (21).

In the current study, we exploit the entire continuum of depression, defined as the number and severity of depressive symptoms a person experiences. We assessed depressive symptoms with one of the most widely used instruments in the general population, namely the Center for Epidemiological Studies Depression (CES-D) scale. This scale assesses the following major dimensions of depression: depressed mood, feelings of guilt and worthlessness, feelings of helplessness and hopelessness, psychomotor retardation, loss of appetite, and sleep disturbance. The CES-D detects cases of MDD with high sensitivity and specificity (22) and has proven to be relatively stable over time (82% of older adults had stable CES-D scores over four measurement rounds in 10 years) (23,24). In addition, a high CES-D score, like a diagnosis of MDD, is associated with cardiovascular disease and mortality (25,26). Moreover, heritability estimates of depressive symptoms, as measured with the CES-D, range from 15% to 34% (27–29).

We present the results of a meta-analysis combining genome-wide association results of depressive symptoms from 17 population-based studies of European ancestry (n = 34,549). In addition, we sought to replicate our findings in five samples that used instruments other than the CES-D to quantify depressive symptoms (n = 16,709). Finally, we performed a combined meta-analysis of all discovery and replication studies that included 51,258 individuals.

**Methods and Materials**

**Discovery Samples**

This discovery set included results from 17 population-based studies comprising a total of 34,549 persons of European descent. The following studies collaborating in the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium (30) in the United States and Europe were included: the Atherosclerosis Risk In Communities 1 and 2 studies (ARIC1 and ARIC2) (31), the Cardiovascular Health Study (CHS) (32), the Framingham Heart Study (FHS) (33,34), and the Rotterdam Study I, II, and III (RS-I, RS-II and RS-III) (35). The following population-based studies joined the discovery analyses: the Baltimore Longitudinal Study of Aging (BLSA) (36); The Erasmus Rucphen Family (ERF) (37) study; the Health, Aging and Body Composition study (Health ABC); the Invecchiare in Chianti (Aging in the Chianti area; InCHIANTI) (38) study; Helsinki Birth Cohort Study (HBCS) (39); Multi-Ethnic Study of Atherosclerosis (MESA) (40); Nurses’ Health Study (NHS) (41); Rush Memory and Aging Project (MAP) (42); Religious Orders Study (ROS) (43), and SardiNIA study (44). All studies were approved by their local institutional review boards and all participants provided written informed consent.

**Phenotype Definition**

Depressive symptoms were measured with the CES-D scale (10-item version [CHS, NHS, Rush MAP, Rush ROS], 11- item version [ARIC1], or 20-item version [ARIC2, BLSA, ...
ERF, FHS, HBCS, Health ABC, InCHIANTI, MESA, RS-I, RS-II, RS-III, SardiNIA). The CES-D scale is designed for use in the general population. All three CES-D versions used here detect the same four latent factors (45): depressed affect, somatic symptoms, positive affect, and interpersonal problems. Each item is scored from 0 to 3 depending on the frequency of the symptoms during the past week. A higher score corresponds to more depressive symptoms. Scores from one examination round per study were used, but CES-D scores have been shown to be relatively stable over time (23,24). In studies with multiple CES-D assessments, the round with the largest number of participants (generally the first examination round) was chosen. Persons with schizophrenia or bipolar disorder were excluded, based on records, interviews, or medication use (these disorders probably have a distinct genetic component). In addition, persons with a Mini-Mental State Examination score < 22, indicative of dementia, were excluded. We included persons with genotype data and depressive symptom score who were aged 40 years and older.

Adjustment for Use of Antidepressants

In the search for common variants for depressive symptoms in a population-based sample, persons using antidepressants, who most likely had depression or depressive symptoms, increase genetic information. We, thus, did not exclude these persons from the analysis, but we chose to adjust their total depressive symptoms score for medication use. However, response to antidepressants is highly variable. In addition, information on compliance is often not available in population-based studies. We therefore used a nonparametric imputation algorithm to adjust the CES-D score for treatment effect. We made two assumptions: the CES-D score of a person using antidepressants is a right-censored value, i.e., the score is lower than the untreated value would be; and persons with a high CES-D score, on average, responded less to their medication than persons with a lower CES-D score. We replaced the score of a person on antidepressants with the mean depressive symptom score of all persons using antidepressants that had the same or a higher depressive symptom score. This procedure was performed separately for men and women and was based on an algorithm used for adjustment of blood pressure for persons on antihypertensive drugs (46). Antidepressant medication was defined by each study separately to account for differences between countries.

Genotyping and Imputation

Genome-wide genotyping was performed by the individual studies on Illumina (Illumina, Inc., San Diego, California) or Affymetrix (Affymetrix, Santa Clara, California) platforms. All studies imputed their genotype data to ~2.5 million single nucleotide polymorphisms (SNPs) to account for the different genotyping platforms. HapMap release 22 CEU (HapMap sample comprised of Utah residents with Northern and Western European ancestry) build 36 was generally used as reference for imputation (two studies used build 35). Genotype and imputation quality control were performed in each study separately. Genotype and quality control procedures for each study can be found in Table S1 in Supplement 1.

Data Analysis

A linear regression was performed on total depressive symptom score, adjusted for age and gender. The distribution of CES-D scores is skewed, but linear regression is fairly robust to nonnormality. Cardiovascular Health Study and Atherosclerosis Risk In Communities additionally adjusted for field study site, NHS for disease status, SardiNIA for self-report versus tester-read and reported answers, and FHS for cohort (offspring, generation 3). Furthermore, FHS used linear mixed effect models to account for familial correlations. In the ERF study, kinship matrix was used to correct for relatedness.
Meta-Analysis

We performed a p value based meta-analysis weighted by sample size. This is a valid approach to account for the different CES-D versions to measure depressive symptoms and for the different distributions of depressive symptoms. The meta-analysis test statistic was computed as follows:

\[ Z_{meta} = \sum \beta_i \times \frac{N_i}{N_{total}} \]

The meta-analysis was performed with METAL (http://www.sph.umich.edu/csg/abecasis/metal/) (47). The beta (\( \beta \)) of each individual study \( i \) was matched to a common coded allele (the minor allele) for each SNP across all studies. Single nucleotide polymorphisms with a minor allele frequency less than 2.5% or an observed to expected variance ratio (imputation quality) less than .30 were excluded on a per study basis. Single nucleotide polymorphisms for which the total sample size was lower than 5000 were removed from the results. Genomic control correction was applied to each study’s results.

Replication

Independent top SNPs with a p value < 1 \times 10^{-5} in the discovery meta-analysis were selected with the clumping function in PLINK (http://pngu.mgh.harvard.edu/purcell/plink/) (48) (\( R^2 < .05, 500 \) kilobase [kb]) for replication in five studies that measured depressive symptoms with other instruments (total \( n = 16,709 \)). Persons included in the replication studies were independent from those in the discovery studies. Although replication with other instruments than the CES-D might introduce some heterogeneity, all instruments measure depressive symptoms. Further, a positive replication would ensure that our top hits are not instrument-dependent.

Age, Gene, Environment Susceptibility–Reykjavik Study (AGES) (49), the ARIC 3 study (31), Monitoring of Trends and Determinants of Cardiovascular Disease/Cooperative Health Research in the Region of Augsburg F3 and F4 (MONICA/KORA F3 and F4) (50), and the Study of Health in Pomerania (SHIP) (51,52) measured depressive symptoms with the Geriatric Depression Scale (GDS), Maastricht Questionnaire, Patient Health Questionnaire (PHQ-9), and the Beck Depression Inventory-II (BDI-II), respectively. The BDI-II, GDS, and PHQ-9 aim to screen for depression and are highly correlated (53,54). The BDI-II is based on the DSM-IV criteria for MDD and comprises 21 items on a scale of 0 to 3 with higher scores indicating more severe depressive symptoms over the past 2 weeks. The PHQ-9 is, like the BDI-II, based on the DSM-IV criteria for MDD, but it consists of nine items on a scale of 0 to 3 to assess depressive symptoms over the past 2 weeks. The GDS was specifically designed to screen for depression in older adults and comprised 15 items answered with “yes” or “no.” The Maastricht Questionnaire (21 items), although designed to measure vital exhaustion, correlates with measures of depressive symptoms (55) and was previously used to assess depressive symptoms (56,57).

Replication was considered significant if the Bonferroni-corrected p value for testing seven SNPs was ≤050 (uncorrected \( p \) value ≤ 7.1 \times 10^{-3}).

Pathway Analysis

Protein ANalysis THrough Evolutionary Relationships (PANTHER) (58) was used to identify and classify biological processes among the SNPs associated with \( p \) values < 10^{-4} from the overall meta-analysis (\( n = 51,258 \)). After SNP selection, SNPs were annotated to genes and/or flanking genes with the SCAN SNP and CNV Annotation Database (http://
Protein ANalysis THrough Evolutionary Relationships then compares this gene list to a reference list (Homo Sapiens gene list from the National Center for Biotechnology Information) using the binomial test. Results were Bonferroni-corrected to account for multiple testing.

Candidate Gene Search

Altogether, 17 SNPs previously reported to be associated to depression were selected: 1 SNP that has been found genome-wide significantly associated with depressive phenotypes after replication (7,59), 4 top SNPs from the largest MDD meta-analysis so far (13), and 12 top SNPs from the only published GWAS that studied a depressive trait continuously (21). Single nucleotide polymorphisms were tested for association in the discovery meta-analysis ($n = 34,549$) and in the overall meta-analysis including all studies that measured depressive symptoms ($n = 51,258$).

Results

Meta-Analysis of Depressive Symptoms

Table 1 shows the characteristics of the study populations. Mean age in the discovery studies ranged between 55.9 and 80.8 years. The percentage of women varied between 44.6% and 100%. In line with the population-based design of the studies, median depressive symptoms scores ranged between 2 and 10 for the CES-D 20-item version. This is well below the cutoff of 16 at which major depression cases in older adults can be identified with high specificity and sensitivity (22). The percentage of persons scoring above this cutoff varied between 4.7% and 27.1%. Distributions of CES-D scores differed between studies and therefore a Z-score based meta-analysis was used to combine the individual study results. Antidepressant use ranged from 3.0% to 14.0%. On average, CES-D scores for persons on antidepressants more than doubled after imputation.

The genomic control inflation factor lambda ($\lambda_{gc}$) for each study ranged between .997 and 1.024. A meta-analysis of 17 studies ($n = 34,549$) with depressive symptoms measured by CES-D was performed (Q-Q and Manhattan plots in Figure S1 in Supplement 1). The total number of SNPs analyzed was 2,391,896. No association reached the prespecified genome-wide significance level of $5 \times 10^{-8}$ for the association with the depressive symptom score. However, we identified 117 SNPs with a $p$ value < $1 \times 10^{-5}$, which included seven independent top SNPs ($R^2 < .05$ in 500 kb, Table 2). The SNP with the lowest $p$ value was rs8020095 ($p = 1.05 \times 10^{-7}$) and maps to an intronic region of GPHN on chromosome 14. Of the seven top SNPs, none had a heterogeneity $p$ value (tested by Cochran’s Q) below .05 in the discovery meta-analysis.

We reran the analysis for the independent top SNPs excluding people on antidepressants; $p$ values of the top SNPs shifted toward one (e.g., rs8020095 $p$ value $1.56 \times 10^{-6}$, rs161645 $p$ value $1.71 \times 10^{-5}$). Adding five points to the total score for people using antidepressants in a subsample (RS-I, RS-II, RS-III, $n = 7925$) resulted in the same top SNPs and similar $p$ values for the top SNPs tested here.

Replication

Table 2 presents the results of the replication analysis and the overall meta-analysis across discovery sample and replication sample. The mean observed to expected variance ratio for the seven top SNPs across all cohorts ranged between .91 and .98 (Table S2 in Supplement 1). In the replication sample, an SNP on chromosome 5 showed an association with depressive symptoms (5q21, rs161645, $p = 9.19 \times 10^{-3}$, Table 2), but this association did not
reach the predefined threshold for multiple testing (corrected for multiple testing $p = .064$). This SNP resides in a gene desert, with the closest gene $NUDT12$ more than 1000 kb away.

In the overall meta-analysis including discovery and replication samples ($n = 51,258$), SNP rs40465 reached genome-wide significance ($p = 4.78 \times 10^{-8}$). This SNP is in high linkage disequilibrium with SNP rs161645 ($R^2 = .80$). Rs40465 had a $p$ value of $2.58 \times 10^{-6}$ in the discovery meta-analysis and a $p$ value of $5.00 \times 10^{-3}$ in the meta-analysis of replication studies. An association plot of the 5q21 region is presented in Figure 1.

In contrast, the strength of the associations of the other top SNPs with depressive symptoms was attenuated, as judged by the $p$ value. All SNPs with a $p$ value < $1 \times 10^{-4}$ from the overall meta-analysis ($n = 51,258$) are presented in Table S3 in Supplement 1.

**Pathway Analysis**

One hundred four functional genes of the 170 genes that were annotated were mapped to biological processes. Relevant processes that were overrepresented among top SNPs ($p$ value < $10^{-4}$) of the overall meta-analysis were neurotransmitter secretion (Bonferroni-corrected $p$ value = $9.84 \times 10^{-3}$), vitamin transport (Bonferroni-corrected $p$ value = .014), and synaptic transmission (Bonferroni-corrected $p$ value = .037). A complete list of biological processes that were significantly overrepresented is presented in Table 3.

**Candidate Gene Search**

None of the 17 tested candidate genes were replicated in the current study (Table S4 in Supplement 1). Nine out of 17 associations had the same direction in our overall meta-analysis as in the published study, and none of the nine was significant (uncorrected for multiple testing).

**Discussion**

In this GWAS of depressive symptoms, we combined the results of 17 population-based studies with 34,549 individuals to find common variants for depressive symptoms. Including the five replication studies, this effort comprised data from 51,258 independent individuals. Of the seven SNPs we attempted to replicate, we found suggestive evidence for the observed association of one SNP in the 5q21 region with depressive symptoms. This region reached genome-wide significance when tested over all studies ($n = 51,258$).

Although evidence shows that depression can be well represented by a continuum of depressive symptoms, we observed a genome-wide significant hit in this large GWAS only when pooling all studies with depressive symptoms. This difficulty of finding signals is in line with GWAS of major depression. Nine GWAS of depression, of which the largest comprised ~6000 MDD cases and ~7000 control subjects, yielded only one genome-wide significant finding (15).

The approach of studying depression on a continuum has the advantage that not only information on extremes is used but that all available information is exploited. Van der Sluis et al. (20) showed that if the phenotypic variation among cases, as well as the variation among control subjects, is used, this greatly increases the power to detect genetic variants. However, studying depression along a continuum in population-based studies implies that many individuals have a low depressive symptoms score and that few persons score high. Therefore, it remains to be validated whether the results presented here are generalizable to clinical depression cases. In addition, the CES-D measures current depressive symptoms and not remitted depressive symptomatology. This introduces false-negatives, but in this population-based approach in which low depressive symptomatology is overrepresented, the
resulting bias would be conservative. Furthermore, the distribution of depressive symptoms differed between cohorts. We therefore performed a \( p \) value based meta-analysis, which is a valid approach, but has the consequence that we cannot draw conclusions on effect sizes.

Differences in depressive symptoms distribution do not impact on the validity of the findings. People with high depressive symptoms are more likely to carry risk variants, but this should not depend on the number of people with a high score. Furthermore, the distribution of \( I^2 \), a measure of heterogeneity (60), of the results combining all samples did not differ from the distribution of \( I^2 \) of the results when samples with low or high depression prevalence were meta-analyzed separately. No excess heterogeneity was observed, which suggests that depressive symptoms can be analyzed linearly. However, some genetic main effects may be more detectable in very homogeneous populations. Observed differences in distributions of depressive symptoms may have resulted from environmental factors, and if these, in turn, interact with specific genetic variants, only very homogeneous studies could also detect a genetic main effect.

Environmental factors, like education level, differed among cohorts. In observational research, one would have controlled for such possible confounders. In genetic studies, confounding by environmental factors is unlikely to occur (61), but controlling for environmental factors can also be done to increase precision, i.e., reduce the variance in depressive symptoms (62). However, environmental factors explain very little variance in depressive symptoms. Therefore, the benefit of performing additional controlled analyses will be negligible and offset by running several models with the risk of multiple testing.

In the current study, depressive symptom scores for people using antidepressants were imputed to take into account the high variability in response to antidepressants. In an analysis of depressive symptoms, people on antidepressants, who most likely had depression or depressive symptoms, are particularly informative. Therefore, excluding this group a priori may have changed the results. In a subsample, the imputation algorithm used in the current study yielded similar results as adding an arbitrary score of five points to the depressive symptom scores of people using antidepressants.

This study was performed in older adults. Cerebrovascular burden and cognitive impairment, which have a relatively high prevalence in old age, are known to be associated with depressive symptoms. In addition, while a high CES-D score indicates depressive symptoms, it can also be suggestive of, for example, anxiety (63). In other words, the level of depressive symptoms is a clinically heterogeneous phenotype. However, the genetic background of clinically heterogeneous phenotypes like anxiety and depression may be more uniform than the clinical presentation suggests (64). In addition, while nongenetic determinants of depression may differ with age, genetic determinants were shown to be stable at different ages (65,66). Therefore, the results presented here are presumably generalizable to younger populations.

We combined results from studies that measured depressive symptoms with instruments other than the CES-D to replicate the association between depressive symptoms and seven independent top SNPs. In an overall meta-analysis, we tested whether any variation introduced by different instruments was offset by the increased power. In the replication effort, one SNP (5q21 region) reached a \( p \) value below .05 but did not pass this threshold when controlling for multiple testing. Another SNP in the 5q21 region, however, reached genome-wide significance when the association across discovery and replication studies was tested \((n = 51,258)\). The 5q21 region resides in a gene desert with the closest gene, \textit{NUDT12}, lying more than 1000 kb away. \textit{NUDT12} has not been previously implicated in psychiatric disorders.
Although we observed suggestive association of the 5q21 region with depressive symptoms, genome-wide significance was observed only after pooling the results of the discovery and replication studies. Also, we could not replicate associations with candidate genes that previously have been reported to be associated with depression. Several explanations are plausible.

A first explanation for these observations is that the top SNPs identified in this study are false-positive findings. However, the discovery set was large and although we did not find any genome-wide significant hits, true hits are expected to be found among the top findings. A pathway analysis on the results of the overall meta-analysis showed that biological processes that play a role in depression were overrepresented among our top hits.

Second, the replication sample was smaller than the discovery sample and may be underpowered to detect true effects with moderate effect sizes, which might have been overestimated in the discovery analysis (winner’s curse). Indeed, we found suggestive evidence of association for only one of seven SNPs, but the direction of association was compatible for five out of seven SNPs.

Third, lack of replication might be related to heterogeneity of the replication phenotype. In the replication approach, we combined the results of studies that measured depressive symptoms with different instruments. Instruments were also administered at different time points across studies. However, the instruments have been reported to be highly correlated (correlations between .77 and .86) and relatively stable genetic determinants over the life span were observed in an Australian Twin study (53,54,65,67,68).

Several other factors can hinder the search for common variants associated with depressive symptoms. Population stratification, for example, can result in false-positive findings. To avoid population stratification, only individuals from European descent were included. Including only individuals from European descent also minimized measurement error caused by cultural differences in responses to the CES-D (69). Other possible explanations are the presence of genetic heterogeneity (70), gene-gene interactions (71), and gene-environment interactions. The interaction between candidate genes and life events has been repeatedly studied for depression (72). However, to study this phenomenon in a genome-wide approach requires much larger data sets (13). In addition, it is suggested that the gain of gene-environment interaction studies over studies of main effects for complex diseases like depression is minimal (73). The study described here focused on common genetic variation, but rare variants or copy number variations not tagged by SNPs might play a role in depression (74,75). Using a larger reference panel, like the haplotypes generated by the 1000 Genomes Project, would have improved the yield of rare variants. Harmonizing imputation reference and imputation tools might have further increased the power of the study to detect associations. Also, not single SNPs, but many SNPs collectively, each with a very small effect, may affect the susceptibility for depressive symptoms (66).

In conclusion, the efforts of a large collaboration to identify common variants associated with depressive symptoms yielded no genome-wide significant hit in the discovery sample. In the replication approach, we found suggestive evidence for a SNP in the 5q21 region. When analyzing the discovery and replication samples, one genome-wide significant hit in this region was observed. Further investigation of the 5q21 region is necessary to verify the association with depressive symptoms and to pinpoint the possible functional variant. Such a future study of depressive symptoms could analyze this phenotype stratified by gender and incorporate longitudinal information with repeated measures of depressive symptoms to provide more power to our search for potential candidate genes.
Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We acknowledge the essential role of the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium in development and support of this manuscript. Cohorts for Heart and Aging Research in Genomic Epidemiology Consortium members include the Netherlands Rotterdam Study, the National Heart, Lung, and Blood Institute’s (NHHLBI) Framingham Heart Study, Cardiovascular Health Study, the NHHLBI’s Atherosclerosis Risk in Communities Study, and the National Institute on Aging’s (NIA) Iceland Age, Gene/Environment Susceptibility Study.

The Age, Gene/Environment Susceptibility Reykjavik Study has been funded by National Institutes of Health (NIH) contract N01-AG-12100, the NIA Intramural Research Program, Hjarlavern (the Icelandic Heart Association), and the Althingi (the Icelandic Parliament).

The Atherosclerosis Risk in Communities Study research is carried out as a collaborative study supported by National Heart, Lung, and Blood Institute contracts N01-HC-55015, N01-HC-55016, N01-HC-55018, N01-HC-55019, N01-HC-55020, N01-HC-55021, and N01-HC-55022 and Grants R01-HL087641, R01-HL093029, and R01-HL70825; National Human Genome Research Institute contract U01-HG004402; and National Institutes of Health contract HHSN268200625226C. We thank the staff and participants of the Atherosclerosis Risk in Communities Study for their important contributions. Infrastructure was partly supported by Grant Number UL1RR025005, a component of the National Institutes of Health and NIH Roadmap for Medical Research.

The Baltimore Longitudinal Study of Aging research was supported entirely by the Intramural Research Program of the NIH, National Institute on Aging.

This Cardiovascular Health Study research was supported by NHLBI contracts N01-HC-85239, N01-HC-85079 through N01-HC-85086, N01-HC-35129, N01 HC-15103, N01 HC-55222, N01-HC-75150, N01-HC-45133 and NHLBI Grants HL080295, HL075366, HL087652, and HL105756 with additional contribution from National Institute of Neurological Disorders and Stroke. Additional support was provided through AG-023629, AG-15928, AG-20098, and AG-027058 from the NIA. See also http://www.chs-nhlbi.org/pi.htm. DNA handling and genotyping was supported, in part, by Clinical and Translational Science Institute Grant UL1RR033176 to the Cedars-Sinai General Clinical Research Center Genotyping core, National Institute of Diabetes and Digestive and Kidney Diseases Grant DK063491 to the Southern California Diabetes Endocrinology Research Center, and the Governors’ Chair in Medical Genetics (JIR).

The Erasmus Rucphen Family (ERF) research was supported through funds from The European Community’s Seventh Framework Programme (FP7/2007-2013), European Network for Genetic and Genomic Epidemiology Consortium, Grant agreement HEALTH-F4-2007- 201413.

The genotyping for the ERF study was supported by European Special Populations Research Network and the European Commission FP6 STRP Grant (018947; LSHG-CT-2006-01947). The ERF study was further supported by Grants from the Netherlands Organisation for Scientific Research, Erasmus MC, the Centre for Medical Systems Biology, and the Netherlands Brain Foundation (HersenStichting Nederland). We are grateful to all participating individuals and their relatives, general practitioners, and neurologists for their contributions and to P. Veraart for her help in genealogy, Jeannette Vergeer for the supervision of the laboratory work, and P. Snijders for his help in data collection.

Framingham Heart Study: The phenotype-genotype association analyses were supported by R01-AG29451. “This research was conducted in part using data and resources from the Framingham Heart Study of the National Heart Lung and Blood Institute of the National Institutes of Health and Boston University School of Medicine. The analyses reflect intellectual input and resource development from the Framingham Heart Study investigators participating in the SNP Health Association Resource project. This work was partially supported by the National Heart, Lung and Blood Institute’s Framingham Heart Study (Contract No. N01-HC-25195) and its contract with Affymetrix, Inc for genotyping services (Contract No. N02-HL-6-4278). A portion of this research utilized the Linux Cluster for Genetic Analysis funded by the Robert Dawson Evans Endowment of the Department of Medicine at Boston University School of Medicine and Boston Medical Center.”

Helsinki Birth Cohort Study has been supported by Grants from the Academy of Finland, the Finnish Diabetes Research Society, Folkhälsoan Research Foundation, Novo Nordisk Foundation, Finska Ljäkareätskabet, Signe and Ane Gyllenberg Foundation, University of Helsinki, European Science Foundation (EUROSTRESS), Ministry of Education, Ahokas Foundation, Emil Aaltonen Foundation, Juho Vainio Foundation, and Wellcome Trust (Grant...
The Health, Aging and Body Composition research was supported by NIA contracts N01AG62101, N01AG62103, and N01AG62106. The genome-wide association study was funded by NIA Grant 1R01AG032098-01A1 to Wake Forest University Health Sciences and genotyping services were provided by the Center for Inherited Disease Research. Center for Inherited Disease Research is fully funded through a federal contract from the National Institutes of Health to The Johns Hopkins University, contract number HHSN268200782096C. This research was supported, in part, by the Intramural Research Program of the NIH, National Institute on Aging. Dr. Yaffe is supported by NIH Grant R01 MH086498.

The Invecchiare in Chianti Study was supported as a targeted project (ICS 110.1RS97.71) by the Italian Ministry of Health, by the U.S. National Institute on Aging (Contracts N01|AG|916413, N01|AG|821336, 263 MD 9164 13, and 263 MD 821336), and, in part, by the Intramural Research Program, National Institute on Aging, National Institutes of Health.

The Multi-Ethnic Study of Atherosclerosis (MESA) SNP Health Association Resource project is conducted and supported by the NHLBI in collaboration with MESA investigators. Support for MESA is provided by contracts N01-HC-95159 through N01-HC-95169 and UL1-RR-024156. Funding for genotyping was provided by NHLBI Contract N02-HL-6-4278 and N01-HC-65226. Funding for this project was also provided by #R01 HL101161.

The Monitoring of Trends and Determinants of Cardiovascular Disease/Cooperative Health Research in the Region of Augsburg studies were financed by the Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany, and supported by Grants from the German Federal Ministry of Education and Research. Furthermore, the research was supported within the Munich Center of Health Sciences as part of Ludwig-Maximilians-University.

The Nurses’ Health Studies are supported by NIH Grants CA 65725, CA87969 (National Cancer Institute), CA49449, CA67262, CA50385, and 5UO1CA098233.

The generation and management of genome-wide association study genotype data for the Rotterdam Study is supported by the Netherlands Organisation of Scientific Research Investments (number 175.010.2005.011, 911-03-012). This study is funded by the Research Institute for Diseases in the Elderly (014-93-015), the Netherlands Genomics Initiative/Netherlands Organisation for Scientific Research project number 050-060-810. The Rotterdam Study is funded by Erasmus MC and Erasmus University, Rotterdam, Netherlands Organization for the Health Research and Development, the Ministry of Education, Culture and Science, the Ministry for Health, Welfare and Sports, the European Commission (Directorate-General XII), and the Municipality of Rotterdam. Henning Tiemeier was supported by the Vidi Grant of Netherlands Organization for the Health Research and Development (2009-017.106.370). Karin Hek was supported by a Grant from BavoEuropoort. We are grateful to the study participants, the staff from the Rotterdam Study, and the participating general practitioners and pharmacists. We thank Pascal Arp, Milla Jamai, Marijn Verkerk, Lizbeth Herrera, and Marjolein Peters for their help in creating the genome-wide association study database, and Dr. Karol Estrada and Maxim V. Struchalin for their support in creation and analysis of imputed data. We thank Dr. Karol Estrada, Dr. Fernando Rivadeneira, Dr. Tobias A. Knoch, Anis Abuseiris, Luc V. de Zeeuw, and Rob de Graaf (Erasmus MC Rotterdam, The Netherlands) for their help in creating GRIMP and BigGRID, MediGRID, and Services@MediGRID/D-Grid (funded by the German Bundesministerium für Forschung und Technology; Grants 01AK 803 A-H, 01 IG 07015 G) for access to their grid computing resources.

The Rush Memory and Aging Project is supported by NIA Grants R01AG15819, R01AG17917, and K08AG34290 and the Translational Genomics Research Institute. The Rush Religious Orders Study is supported by NIA Grants P30AG10161, R01AG15819, R01AG30146, and K08AG34290 and the Translational Genomics Research Institute. We thank the study participants and the staff of the Rush Alzheimer’s Disease Center. Joshua Shulman was additionally supported by a Career Award for Medical Scientists from Burroughs Wellcome Fund.

The SardiNIA research was supported, in part, by the Intramural Research Program of the NIH, National Institute on Aging. Funding was also provided through contract NO1-AG-1-2109 from the NIA-NIH.

Study of Health in Pomerania is part of the Community Medicine Research net of the University of Greifswald, Germany, which is funded by the Federal Ministry of Education and Research (Grant no. 01ZZ9603, 01ZZ0103, and 01ZZ0403), the Ministry of Cultural Affairs, and the Social Ministry of the Federal State of Mecklenburg-West Pomerania. Genome-wide data have been supported by the Federal Ministry of Education and Research (Grant no. 03ZIK012) and a joint Grant from Siemens Healthcare, Erlangen, Germany, and the Federal State of Mecklenburg-West Pomerania. The University of Greifswald is a member of the Center of Knowledge Interchange program of the Siemens AG. This work was also funded by the German Research Foundation (DFG: GR 1912/5-1), Federal Ministry of Education and Research Germany, the Humboldt Foundation, and the German Research Foundation.
Study of Health in Pomerania: To HJG German Research Foundation; Federal Ministry of Education and Research Germany; speakers honoraria from Bristol-Myers Squibb, Eli Lilly, Novartis, Eisai, Wyeth, Pfizer, Boehringer Ingelheim, and Servier; and travel funds from Lundbeck, Janssen-Cilag, Eli Lilly, Novartis, AstraZeneca, and SALUS-Institute for Trend-Research and Therapy Evaluation in Mental Health. To HV research grants by Sanofi-Aventis, Biotronik, the Humboldt Foundation, the Federal Ministry of Education and Research (Germany), and the German Research Foundation.

References


Biol Psychiatry. Author manuscript; available in PMC 2014 April 01.

Figure 1.
Association results in the 5q21 region. Summary of the association of single nucleotide polymorphisms (SNPs) on chromosome 5 (base 103,500,000 to 104,500,000) with depressive symptoms from the overall meta-analysis (n = 51,258). The SNP with the strongest association (rs40465) is highlighted in blue and its corresponding p value is given. Other SNPs are colored according to their degree of linkage disequilibrium (LD) with rs40465, ranging from high LD (orange, $R^2$ > 1.0) to low LD (white, $R^2$ < .2). cM, centimorgan; kb, kilobase; Mb, megabase.
### Study Sample Characteristics of Discovery and Replication Samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Instrument</th>
<th>n</th>
<th>Mean (SD)</th>
<th>Median (Range)</th>
<th>≥16 yrs %</th>
<th>Antidepressant Users %</th>
<th>Mean Age (SD)</th>
<th>Female %</th>
<th>Current Smokers %</th>
<th>Level 01 %</th>
<th>Level 2 %</th>
<th>Level 3 %</th>
<th>Level 4 %</th>
<th>Level 56 %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discovery Studies</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ARIC 1</td>
<td>CES-D 11</td>
<td>393</td>
<td>3.80 (3.57)</td>
<td>3 (0–18)</td>
<td>9.92</td>
<td>14.0</td>
<td>72.7 (5.46)</td>
<td>59.5</td>
<td>19.6</td>
<td>2.0</td>
<td>8.1</td>
<td>35.4</td>
<td>7.9</td>
<td>46.6</td>
</tr>
<tr>
<td>ARIC 2</td>
<td>CES-D 20</td>
<td>614</td>
<td>8.52 (7.41)</td>
<td>6 (0–34)</td>
<td>16.1</td>
<td>11.1</td>
<td>71.0 (5.60)</td>
<td>49.7</td>
<td>19.7</td>
<td>3.1</td>
<td>8.3</td>
<td>34.7</td>
<td>11.7</td>
<td>42.2</td>
</tr>
<tr>
<td>BLSA</td>
<td>CES-D 20</td>
<td>764</td>
<td>6.90 (6.5)</td>
<td>5 (0–55)</td>
<td>8.51</td>
<td>NA</td>
<td>71.6 (13.8)</td>
<td>44.6</td>
<td>3.0</td>
<td>.4</td>
<td>1.5</td>
<td>11.0</td>
<td>12.4</td>
<td>74.8</td>
</tr>
<tr>
<td>CHS</td>
<td>CES-D 10</td>
<td>3155</td>
<td>4.27 (4.29)</td>
<td>3 (0–26)</td>
<td>11.3</td>
<td>3.11</td>
<td>72.2 (5.29)</td>
<td>61.2</td>
<td>11.0</td>
<td>2.5</td>
<td>12.3</td>
<td>38.6</td>
<td>9.3</td>
<td>37.2</td>
</tr>
<tr>
<td>ERF</td>
<td>CES-D 20</td>
<td>1297</td>
<td>12.7 (10.9)</td>
<td>10 (0–59)</td>
<td>27.1</td>
<td>8.20</td>
<td>55.9 (10.1)</td>
<td>56.7</td>
<td>43.2</td>
<td>40.4</td>
<td>42.5</td>
<td>13.6</td>
<td>NA</td>
<td>3.5</td>
</tr>
<tr>
<td>FHS</td>
<td>CES-D 20</td>
<td>4956</td>
<td>7.25 (8.21)</td>
<td>4 (0–53)</td>
<td>10.3</td>
<td>10.4</td>
<td>56.1 (10.5)</td>
<td>53.3</td>
<td>14.7</td>
<td>5.0</td>
<td>3.1</td>
<td>32.2</td>
<td>24.9</td>
<td>39.2</td>
</tr>
<tr>
<td>HABC</td>
<td>CES-D 20</td>
<td>1654</td>
<td>4.93 (5.78)</td>
<td>3 (0–43)</td>
<td>4.70</td>
<td>3.60</td>
<td>73.8 (2.80)</td>
<td>47.1</td>
<td>6.4</td>
<td>11.9</td>
<td>NA</td>
<td>34.4</td>
<td>53.6</td>
<td>NA</td>
</tr>
<tr>
<td>InCHIAN</td>
<td>CES-D 20</td>
<td>542</td>
<td>11.8 (8.24)</td>
<td>10 (0–46)</td>
<td>24.6</td>
<td>3.40</td>
<td>70.4 (9.85)</td>
<td>52.8</td>
<td>18.5</td>
<td>73.5</td>
<td>11.2</td>
<td>7.3</td>
<td>4.6</td>
<td>3.4</td>
</tr>
<tr>
<td>RS1</td>
<td>CES-D 20</td>
<td>3791</td>
<td>4.86 (7.35)</td>
<td>2 (0–52)</td>
<td>7.30</td>
<td>3.80</td>
<td>72.7 (7.21)</td>
<td>58.5</td>
<td>16.4</td>
<td>31.4</td>
<td>29.0</td>
<td>29.8</td>
<td>NA</td>
<td>9.8</td>
</tr>
<tr>
<td>RS2</td>
<td>CES-D 20</td>
<td>2093</td>
<td>5.81 (7.90)</td>
<td>3 (0–48)</td>
<td>9.70</td>
<td>5.00</td>
<td>64.8 (8.03)</td>
<td>54.5</td>
<td>19.6</td>
<td>21.6</td>
<td>35.6</td>
<td>27.1</td>
<td>NA</td>
<td>15.7</td>
</tr>
<tr>
<td>RSII</td>
<td>CES-D 20</td>
<td>1386</td>
<td>9.58 (8.68)</td>
<td>7 (0–53)</td>
<td>19.4</td>
<td>4.70</td>
<td>63.4 (2.86)</td>
<td>59.7</td>
<td>23.0</td>
<td>33.0</td>
<td>18.4</td>
<td>26.0</td>
<td>NA</td>
<td>22.5</td>
</tr>
<tr>
<td>HBCS</td>
<td>CES-D 20</td>
<td>2423</td>
<td>6.93 (6.87)</td>
<td>5 (0–50)</td>
<td>10.0</td>
<td>12.2</td>
<td>62.7 (10.2)</td>
<td>52.2</td>
<td>11.4</td>
<td>1.6</td>
<td>3.4</td>
<td>16.5</td>
<td>28.4</td>
<td>50.1</td>
</tr>
<tr>
<td>MESA</td>
<td>CES-D 10</td>
<td>5891</td>
<td>6.36 (4.50)</td>
<td>6 (0–26)</td>
<td>15.9</td>
<td>13.3</td>
<td>71.7 (6.70)</td>
<td>100</td>
<td>5.5</td>
<td>0</td>
<td>0</td>
<td>0.0</td>
<td>72.6</td>
<td>27.4</td>
</tr>
<tr>
<td>RS3</td>
<td>CES-D 20</td>
<td>2041</td>
<td>6.32 (8.22)</td>
<td>3 (0–53)</td>
<td>9.90</td>
<td>6.90</td>
<td>56.0 (5.67)</td>
<td>56.1</td>
<td>22.4</td>
<td>9.8</td>
<td>35.0</td>
<td>28.4</td>
<td>NA</td>
<td>26.8</td>
</tr>
<tr>
<td>Rush MA</td>
<td>CES-D 10</td>
<td>825</td>
<td>1.38 (1.75)</td>
<td>1 (0–8)</td>
<td>20.1</td>
<td>13.6</td>
<td>80.8 (6.53)</td>
<td>73.0</td>
<td>2.4</td>
<td>1.7</td>
<td>27.4</td>
<td>19.9</td>
<td>42.8</td>
<td>8.2</td>
</tr>
<tr>
<td>Rush RO</td>
<td>CES-D 10</td>
<td>778</td>
<td>1.10 (1.51)</td>
<td>1 (0–8)</td>
<td>13.9</td>
<td>9.00</td>
<td>75.5 (7.24)</td>
<td>66.5</td>
<td>2.1</td>
<td>1.3</td>
<td>5.4</td>
<td>3.1</td>
<td>46.0</td>
<td>44.2</td>
</tr>
<tr>
<td>SardiNIA</td>
<td>CES-D 20</td>
<td>1438</td>
<td>11.9 (8.20)</td>
<td>10 (0–53)</td>
<td>25.2</td>
<td>3.00</td>
<td>58.0 (11.4)</td>
<td>59.5</td>
<td>NA</td>
<td>28.9</td>
<td>50.3</td>
<td>16.1</td>
<td>NA</td>
<td>4.8</td>
</tr>
<tr>
<td>Replication Studies</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AGES-RS</td>
<td>GDS</td>
<td>2855</td>
<td>2.58 (2.26)</td>
<td>2 (0–15)</td>
<td>9.92</td>
<td>13.8</td>
<td>76.4 (5.46)</td>
<td>58.0</td>
<td>12.7</td>
<td>22.1</td>
<td>16.8</td>
<td>NA</td>
<td>33.3</td>
<td>27.8</td>
</tr>
<tr>
<td>ARIC 3</td>
<td>MQ</td>
<td>8918</td>
<td>10.2 (8.79)</td>
<td>8 (0–42)</td>
<td>9.39</td>
<td>4.04</td>
<td>57.2 (5.67)</td>
<td>52.7</td>
<td>23.8</td>
<td>4.8</td>
<td>10.2</td>
<td>36.4</td>
<td>9.2</td>
<td>39.4</td>
</tr>
<tr>
<td>MK F3</td>
<td>PHQ-9</td>
<td>1433</td>
<td>3.52 (3.54)</td>
<td>3 (0–26)</td>
<td>6.80</td>
<td>NA</td>
<td>60.5 (9.13)</td>
<td>51.3</td>
<td>14.3</td>
<td>12.1</td>
<td>56.4</td>
<td>17.6</td>
<td>8</td>
<td>13.1</td>
</tr>
<tr>
<td>MK F4</td>
<td>PHQ-9</td>
<td>1807</td>
<td>3.36 (3.3)</td>
<td>3 (0–27)</td>
<td>5.50</td>
<td>NA</td>
<td>60.9 (8.85)</td>
<td>51.5</td>
<td>14.6</td>
<td>10.0</td>
<td>52.4</td>
<td>22.6</td>
<td>1.1</td>
<td>14.0</td>
</tr>
<tr>
<td>SHIP</td>
<td>BDI-II</td>
<td>1696</td>
<td>6.44 (7.11)</td>
<td>4 (0–58)</td>
<td>8.90</td>
<td>NA</td>
<td>59.4 (11.6)</td>
<td>51.4</td>
<td>25.5</td>
<td>5.1</td>
<td>3</td>
<td>60.4</td>
<td>15.9</td>
<td>18.4</td>
</tr>
</tbody>
</table>

**Notes:**
- ARIC1, ARIC2, ARIC3, RSI, RSII, RSIII, MK F3, and MK F4 included unique individuals.
- AGES-RS, Age, Gene, Environment Susceptibility–Reykjavik Study; ARIC, Atherosclerosis Risk in Communities study; BDI-II, Beck Depression Inventory-II; BLSA, Baltimore Longitudinal Study of Aging; CES-D, Center for Epidemiologic Studies Depression scale; CHS, Cardiovascular Health Study; ERF, Erasmus Rucphen Family study; FHS, Framingham Heart Study; GDS, Geriatric Depression

**Table 1**

Risk Psychiatry. Author manuscript; available in PMC 2014 April 01.
<table>
<thead>
<tr>
<th>Scale; HABC, Health, Aging and Body Composition study; HBCS, Helsinki Birth Cohort Study; InCHIANTI, Invecchiare in Chianti; MESA, Multi-Ethnic Study of Atherosclerosis; MK, Monitoring of trends and determinants of cardiovascular disease/cooperative health research in the region of Augsburg (MONICA/KORA); MQ, Maastricht Questionnaire; NA, not applicable; NHS, Nurses Health Study; PHQ-9, Patient Health Questionnaire-9 items; RS, Rotterdam Study; Rush MAP, Rush Memory and Aging Project; Rush ROS, Rush Religious Orders Study; SardiNIA, SardiNIA study; SHIP, Study of Health In Pomerania; SD, standard deviation.</th>
</tr>
</thead>
<tbody>
<tr>
<td>aCutoff for screen positives was 9 for ARIC1, 8 for CHS, 9 for NHS, 3 for Rush MAP and Rush ROS, 6 for AGES-RS, 24 for ARIC3, and 17 for SHIP.</td>
</tr>
<tr>
<td>bLevel 0: preprimary education; level 1: primary education or first stage of basic education; level 2: lower secondary education or second stage of basic education; level 3: (upper) secondary education; level 4: postsecondary nontertiary education; level 5: first stage of tertiary education; level 6: second stage of tertiary education.</td>
</tr>
</tbody>
</table>
### Table 2

Meta-Analysis Results of CES-D Depressive Symptom Score in Discovery Studies, Replication of Results in Studies that Measured Depressive Symptoms with Other Instruments, and Overall Meta-Analysis of All Studies

<table>
<thead>
<tr>
<th>SNP⁶</th>
<th>Chr</th>
<th>Position (Base Pair)</th>
<th>Closest Gene</th>
<th>Distance (Base Pair)</th>
<th>Allele</th>
<th>MAF</th>
<th>Overall Direction (Per Study)</th>
<th>p Value</th>
<th>Overall Direction (Per Study)</th>
<th>p Value</th>
<th>Overall Direction (Per Study)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs8020095</td>
<td>14</td>
<td>66,523,611</td>
<td>GPHN</td>
<td>intron</td>
<td>A/G</td>
<td>.17</td>
<td>+ ++ + + + + + + + + + + + ?</td>
<td>1.05e-07</td>
<td>− ? − − +</td>
<td>.79</td>
<td>+</td>
<td>3.04e-06</td>
</tr>
<tr>
<td>rs8038316</td>
<td>15</td>
<td>52,560,732</td>
<td>UNC13C</td>
<td>intron</td>
<td>A/G</td>
<td>.05</td>
<td>− − − − − − − − − − − + + +</td>
<td>1.24e-06</td>
<td>− − − − +</td>
<td>.42</td>
<td>−</td>
<td>9.64e-06</td>
</tr>
<tr>
<td>rs161645</td>
<td>5</td>
<td>104,097,816</td>
<td>NUDT12</td>
<td>1,171,427</td>
<td>A/G</td>
<td>.34</td>
<td>+ + + + + + + + + + + + + +</td>
<td>2.32e-06</td>
<td>+ (+ + +)</td>
<td>9.19e-03</td>
<td>+</td>
<td>8.39e-06</td>
</tr>
<tr>
<td>rs357282</td>
<td>5</td>
<td>38,904,792</td>
<td>OSMR</td>
<td>intron</td>
<td>T/G</td>
<td>.13</td>
<td>+ + + + + + + + + + + + +</td>
<td>7.56e-06</td>
<td>+ (− + +)</td>
<td>.87</td>
<td>+</td>
<td>1.60e-04</td>
</tr>
<tr>
<td>rs4653363</td>
<td>1</td>
<td>223,662,313</td>
<td>LBR</td>
<td>intron</td>
<td>A/G</td>
<td>.16</td>
<td>− − − − − − − − − − − − − +</td>
<td>8.14e-06</td>
<td>+ (+ + +)</td>
<td>.55</td>
<td>−</td>
<td>8.89e-04</td>
</tr>
<tr>
<td>rs4594522</td>
<td>20</td>
<td>30,718,665</td>
<td>COMMD7</td>
<td>35,508</td>
<td>C/T</td>
<td>.36</td>
<td>− − − − − − − − − − − − − − +</td>
<td>9.29e-06</td>
<td>− (+ +)</td>
<td>.80</td>
<td>−</td>
<td>1.56e-04</td>
</tr>
<tr>
<td>rs13137117</td>
<td>4</td>
<td>94,673,387</td>
<td>GRID2</td>
<td>intron</td>
<td>T/A</td>
<td>.25</td>
<td>+ + + + + + + + + + + + + +</td>
<td>9.77e-06</td>
<td>+ (+ + +)</td>
<td>.97</td>
<td>+</td>
<td>2.63e-04</td>
</tr>
</tbody>
</table>


⁶Independent SNPs with a p value < 1 × 10⁻⁵ in the discovery meta-analysis. The total n for SNP rs8020095 was 40,902, for rs8038316 was 48,103, for rs161645 was 49,820, and for the other SNPs was 51,258. The mean observed versus expected variance ratio (measure of imputation quality) for imputed SNPs ranged between .91 and .99. Table S2 in Supplement 1 includes this information detailed per SNP.

⁷Supporting SNPs: number of SNPs in linkage disequilibrium with the top SNP (R² > .8), with a p value < 10⁻⁴.

Lowest p value of the overall meta-analysis p = 4.78 × 10⁻⁸ for SNP rs40465 (G/T) that is in linkage disequilibrium (R² = .80) with rs161645, discovery p = 2.58 × 10⁻⁶ (+++ + + + + + + + +), replication p = 5.00 × 10⁻³ (+ + +).

Heterogeneity p value < .05.
Table 3
Pathway Analysis

<table>
<thead>
<tr>
<th>Biological Process</th>
<th>NCBI</th>
<th>Observed</th>
<th>Expected</th>
<th>Over/Under</th>
<th>Adjusted p Value$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neurotransmitter Secretion</td>
<td>346</td>
<td>6</td>
<td>1.81</td>
<td>+</td>
<td>9.84e-03</td>
</tr>
<tr>
<td>Vitamin Transport</td>
<td>95</td>
<td>3</td>
<td>.50</td>
<td>+</td>
<td>.014</td>
</tr>
<tr>
<td>Protein Metabolic Process</td>
<td>3240</td>
<td>26</td>
<td>16.92</td>
<td>+</td>
<td>.015</td>
</tr>
<tr>
<td>Synaptic Transmission</td>
<td>594</td>
<td>7</td>
<td>3.10</td>
<td>+</td>
<td>.037</td>
</tr>
<tr>
<td>Transport</td>
<td>2857</td>
<td>22</td>
<td>14.92</td>
<td>+</td>
<td>.038</td>
</tr>
<tr>
<td>Vesicle-Mediated Transport</td>
<td>1160</td>
<td>11</td>
<td>6.06</td>
<td>+</td>
<td>.040</td>
</tr>
<tr>
<td>Cation Transport</td>
<td>621</td>
<td>7</td>
<td>3.24</td>
<td>+</td>
<td>.045</td>
</tr>
<tr>
<td>Cell-Cell Signaling</td>
<td>1331</td>
<td>12</td>
<td>6.95</td>
<td>+</td>
<td>.045</td>
</tr>
<tr>
<td>Protein Transport</td>
<td>1646</td>
<td>14</td>
<td>8.60</td>
<td>+</td>
<td>.048</td>
</tr>
<tr>
<td>Intracellular Protein</td>
<td>1646</td>
<td>14</td>
<td>8.60</td>
<td>+</td>
<td>.048</td>
</tr>
</tbody>
</table>

Enrichment of biological processes among the top results (overall meta-analysis $p$ value < $10^{-4}$) was statistically tested with a binomial test.

NCBI: number of genes in a biological process (reference). Observed: number of genes that belong to a biological process among the GWAS results. Expected: expected number of genes that belong to a biological process in the GWAS results. Over/under: overrepresentation or underrepresentation of the genes in the results.

GWAS, genome-wide association studies; NCBI, National Center for Biotechnology Information.

$^a$ A Bonferroni-correction was applied to correct for multiple testing.